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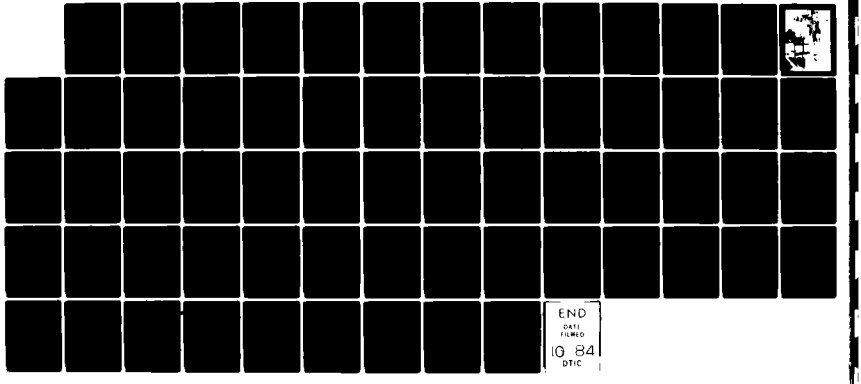

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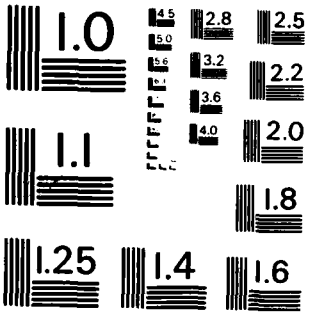

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ASSESSMENT OF ANTIRADIATION DRUG EFFECTIVENESS TO FISSION NEUTRON IRRADIATION

ANNUAL REPORT

CURTIS P. SIGDESTAD, Ph.D.

September, 1982

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-81-C-1070

University of Louisville School of Medicine
Louisville, Kentucky 40292

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SUMMARY

This report describes the assays of various compounds for their toxicity and anti-radiation efficacy following exposure to either Co-60 or fission neutron irradiation. The chemical covered in this report are: WR 347, WR 1065, WR 2529, WR 2721, WR 3689, WR 44923, WR 109342, WR 151327 and WR 168643.

The drugs and their respective dose modification factors (DMF) for fission neutron gastrointestinal lethality (LD50-6) following intraperitoneal administration are, in decreasing order of effectiveness: WR 44923 (1.77), WR 2529 (1.47), WR 1065 (1.42), WR 2721 (1.39), WR 16843 (1.23). Following per os (P. O.) administration of the drug, the DMF's for the LD50-6 are: WR 109342 (1.47), WR 3689 (1.36), and WR 168643 (1.31).

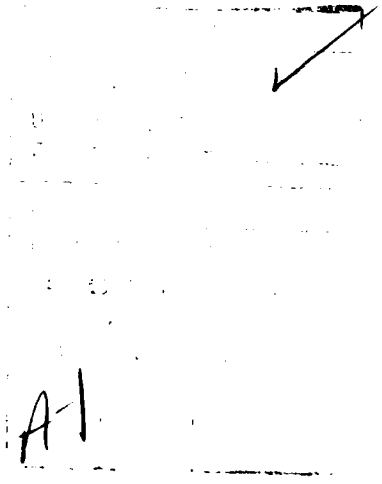
For hematopoietic neutron radiation lethality (LD50-30) the DMF's are: following i.p. administration, WR 2529 (1.40), WR 151327 (1.34), WR 168643 (1.25), WR 44923 (1.22), WR 2721 (1.20), WR 1065 (1.04); following P. O. administration, WR 168643 (1.38), WR 109342 (1.21), WR 3689 (1.04).

Using an intestinal microcolony assay system the following drugs provided the listed DMF's against neutron radiation after i.p. injection: WR 3689 (1.24), WR 2721 (1.15), WR 44923 (1.14), and WR 347 (1.05).

The protective effects against neutron radiation using an endogenous spleen colony assay and i.p. administration were: WR 3689 (1.18), WR 2529 (1.15), WR 2721 (1.10), WR 44923 (1.02) and WR 347 (0.94).

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Publication No. 78-23, Revised 1978).



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INTRODUCTION

The efficacy of sulphydryl compounds as radioprotective agents was first demonstrated by Patt in 1949 (1). Subsequent to these studies, many compounds have been tested for their ability to protect against the effects of ionizing radiation. The decarboxylated cysteine derivative, cysteamine (mercaptoethylmine, MEA, WR 347) was found to be the best protector in the sulphydryl class, giving dose reduction factors (DRF) of approximately 1.3 for intestinal death (2) and 1.8 for hematopoietic death (3). In 1959 Akerfeldt (4) reported the synthesis of a thiophosphate derivative of cysteamine which was characterized by a phosphate group covering the sulphydryl. These phosporothiotic acids were shown to provide significant increases in radioprotection as compared to the compounds containing sulphydryl groups alone (5,6). Further synthesis and screening of phosphorothiotic acids demonstrated the most effective radioprotector to be WR 2721 (7). It is not only less toxic than cysteamine (8), but it also protects irradiated skin and bone marrow preferentially over tumor (9) and provides differential protection in several other normal tissue-tumor systems (10,11,12,13). However, there are problems of toxicity and less than adequate protection of some dose-limiting critical organs, such as kidney, lung, and central nervous system (8,14).

More recently, other phosphorothiotic compounds have been synthesized which may provide either decreased toxicity or increased protection as compared to WR 2721. Some of these drugs, such as cysteamine phosphate (WR 638) and WR 77913 have shown radioprotection comparable to that of WR 2721 in the small intestine (2,15,16). Davidson (17) has recently reported that WR 3689 is better tolerated and has better protective activity in mice than WR 2721. The present study extends the number of thiophosphate compounds investigated as potential radioprotective agents against either Co-60 gamma radiation or fission neutron radiation.

MATERIALS AND METHODS

1. Animals

The animals used in all experiments were male C57/Bl-6 mice (Charles River), 70-77 days of age at the time of exposure to drugs and/or radiation. Prior to beginning any experiment the mice were allowed one week to adapt to the local animal care facility's environmental conditions. They were kept either on wood-chip bedding in plastic mouse boxes (28 x 17 x 12.5 cm) with stainless steel wire tops (five animals per box) or in hanging drawer cages (30 x 30 x 30 cm). The mice were maintained on standard mouse chow (Purina) and HCl acid water (15 ppm) ad libitum. The acid water served to control intestinal flora, the growth of which could serve as a source of error in gastrointestinal lethality experiments. This was a change from the previous use of chlorinated water, which controlled bacterial growth to the same extent but had to be replaced more often (approximately

every two days versus every three or four days for the acid water) due to the sublimation of the chlorine. The animal room was maintained at 22 degrees C and with a 12/12 lour light-dark cycle (lights on at 0600 hours Eastern Standard Time and lights off at 1800 hours EST).

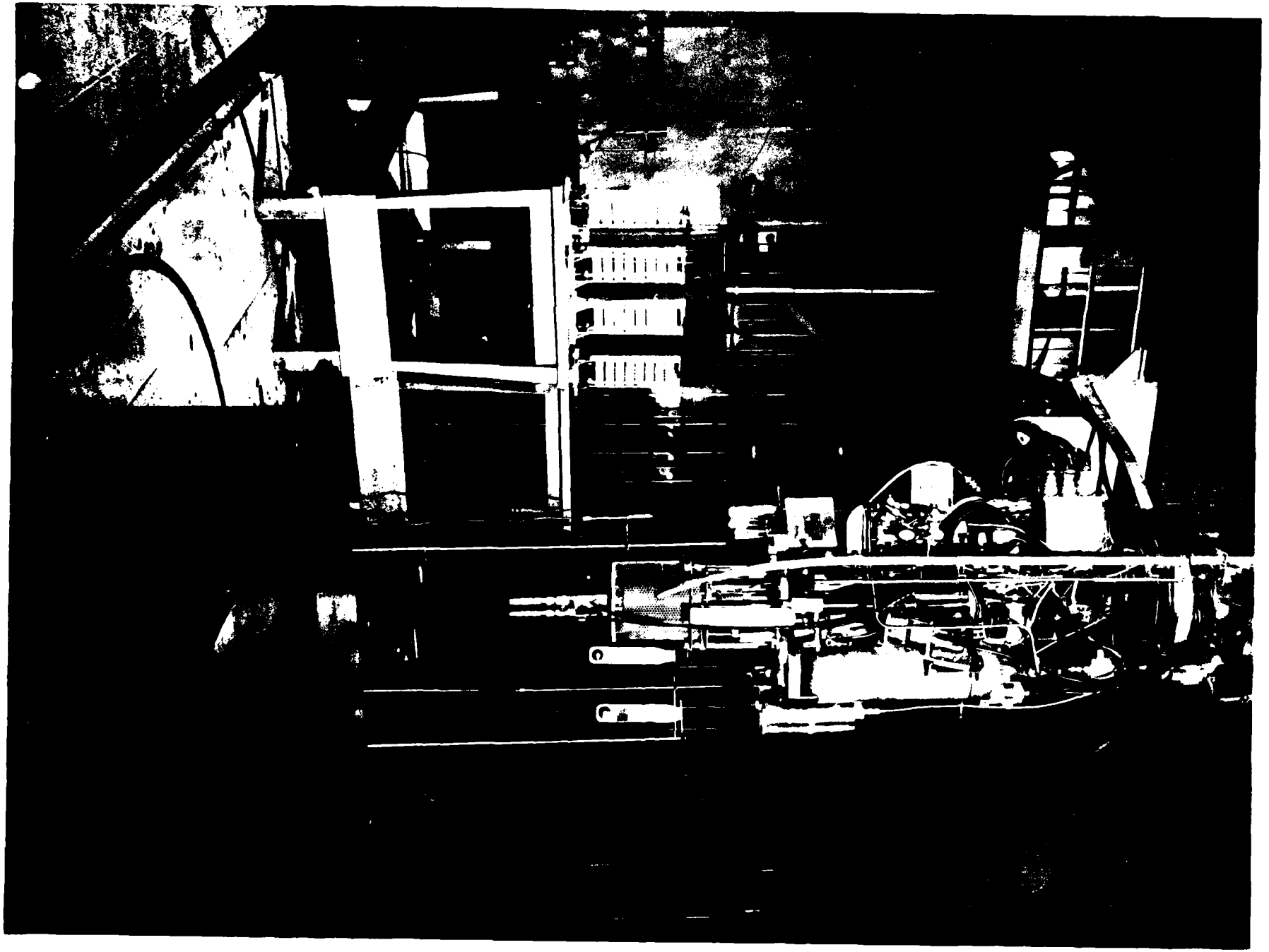
2. Low LET Radiation

In all low LET irradiation procedures performed until September 1, 1981, mice were exposed to whole-body gamma radiation with a cobalt-60 teletherapy unit (Picker C-10000). A 20 x 20 centimeter field was used with a source-to-subject distance (SSD) of 95 centimeters. The dose rate varied slightly from experiment to experiment, due to radioactive decay, but was generally in the 95-100 rads/minute range. An average exposure rate was determined with a thimble changer and a Victoreen condenser R-meter. A plexiglass container was used to hold the ten mice which were used at each dose level. After September 1, 1981, due to the change of the project locale frm the Radiation Center to the J. Graham Brown Cancer Center, low LET irradiations were done with a AECL Therac 780 cobalt teletherapy unit. A 35 x 35 centimeter field was used with a SSD of 80 centimeters. This provided a dose rate of approximately 200 rads/minute. Dosimetry was performed as described above.

3. High LET Radiation

Fission neutron facilities for mouse irradiation were furnished by the Health Physics Research Reactor (DOSAR) at Oak Ridge National Laboratory. The reactor facility has been described in some detail (18). The fission spectrum has a peak energy of 0.9 MeV with a mean energy of 1.2 MeV. In all irradiation procedures performed until May 11, 1982, the mice were irradiated in 3.0 mm thick nylon tubes. It was determined that these nylon tubes attenuated the neutrons by a factor of 0.972 and appropriate adjustments were made in the dosimetry. Beginning May 11, 1982, the mice were irradiated in 1.6 mm thick butyrate tubes and holders of our own design. These tubes and holders were substantially easier to handle and in addition provided a less stressful irradiation environment for the mice. Dosimetry measurements made with the new tubes indicated that there is no measurable attenuation due to their use. The tubes were placed in lucite racks (for the nylon tubes) or oak racks (for the butyrate tubes) two meters or more from the unshielded core. Fig. 1 shows the irradiation setup with nylon tubes in racks on tables at various distances from the reactor. The power level was set at 2 KW with a dose rate of about 30-40 rads/min. Gamma contamination usually amounted to about 15% of the dose for the irradiation protocols used in these experiments. A good discussion of the dose and LET distribution in small animals using this reactor was presented by Willhoit and Jones in 1970 (19). Sigdestad (20) has described the RBE for this radiation procedure.

Fig. 1 - Fission Neutron Radiation Procedure



4. Radioprotective Compounds

The radioprotective compounds used throughout this investigation were: WR 347, WR 2529, WR 2721, WR 3689, WR 44923, WR 109342, WR 151327, WR 168643, and WR 176542. Pertinent information concerning these drugs is given in Table 1. All drugs were either water-soluble or formed fine suspensions in water after vortex mixing which were suitable for intraperitoneal (i.p.) or per os (p.o.) administration. The homogeneity of the solutions or suspensions was demonstrated by the lineal results of the drug toxicity experiments.

Drugs were administered 30 minutes prior to gamma radiation exposure following an i.p. injection and 30-45 minutes prior to neutron irradiation after i.p. administration. For oral administration the drugs were given 45 minutes prior to low LET radiation or neutron radiation although the time of drug administration to irradiation was more variable in the neutron radiation due to reactor operation constraints. There were delays in the time from drug administration to reactor-on time due to the fact that the mice were prepared in the reactor control building, transported to the reactor site, and irradiated following the standard muster call, badge check, and reactor start-up procedure.

5. Lethality Experiments - Drugs

Drugs were administered to the mice either intraperitoneally (i.p.) or per os (p.o.). Intraperitoneal injections were performed with one ml tuberculin syringes and 25 gauge 5/8 needles. Oral administrations of the drugs were done with a six cm x 1.5 mm esophageal cannula which allowed deposition of the compounds in the gastric compartment. Ten mice per dose group were used in each lethality experiment and lethalities were recorded for ten days after drug administration, although no deaths occurred later than two days post-injection with any of the drugs used in these experiments. The probit analysis method of Finney (21) was used to calculate the lethal drug dose for 50% of the population (the LD-50).

6. Lethality Experiments - Radiation

Four to ten mice were used in each radiation dose group. Where 0% of 100% lethal response was expected, fewer numbers of animals were used in order to ensure higher efficiency in numbers of mice used per significantly-weighted data point without altering the reliability of the probit response curve. This was able to be done because in probit analysis less weight or significance is given to responses that are at or near either 0% or 100%, no matter how many organisms are used in the assay at these response points.

Lethalities were recorded each day for thirty days following radiation exposure and were scored either as LD-50(6) (lethalities

occurring within the first six days post-irradiation) or LD-50(30) (lethalities occurring within 30 days post-irradiation). The LD-50(6) is usually regarded as a measure of death due to damage to the gastrointestinal system while death occurring in the seven to thirty day time-frame is due to damage to the bone marrow and blood-forming system (hematopoietic death. The DMF's for lethality were calculated at the LD-50 value as follows: DMF = LD-50 treated/LD-50 untreated.

7. Intestinal Crypt Microcolony Survival

Wither's microcolony assay was used to determine crypt survival in the intestine (25). Three and one-half days following irradiation a section of the jejunem 1-2 cm distal to the ligament of Treitz was excised from each treated mouse and placed in formol saline for fixation. Following fixation the specimens were histologically processed and thin (3-5 um) transverse sections made and mounted on microscope slides. The tissue was then stained with hematoxylin and eosin and microscopically examined to count the surviving cryptal microcolonies. In each transverse section the number of regenerating crypts was examined microscopically and scored (crypts/circumference) and the proportion of crypts destroyed by radiation ascertained. From these data dose-survival curves for control and drug-treated animals were obtained and the survival curve parameters compared by multiple-regression analysis. In the normal jejunem of these mice there were about 137 crypts per circumference. Therefore fewer than 137 crypts were counted in sections which had been irradiated. Crypts were plotted as a function of dose to give a crypt survival curve. However, in order to obtain a cell survival curve one must assume independent survival of cells and that the survival of one cell is sufficient to repopulatet the surviving crypt that is counted three and one-half days following irradiation. Using Poisson statistics, the average number of surviving cells per circumference were calculated as follows: If the number of cells surviving per circumference is n, the average crypt survival per jejunal circumference is $137(-\ln((137-n)/137))$.

8. Endogenous Spleen Colony Assay

This assay was based on the method described by Smith et al. (26). Endogenous spleen colony forming units were determined by removing the spleens of irradiated mice 10 days following exposure, fixing them in Bouin's fixative, and utliziing a dissecting microscope at 20X magnification to count the visible nodules on the spleen. In order to obtain numbers of colonies which would regress linearly with dose a modified logarithmic transform was applied to the colony counts. The following transformation was used: $Y = (\text{antilog}(S\log(x-1)/n))-1$, where x is the individual spleen colony count and n is the number of counts made. The transformed number of nodule (colonies) were then plotted as a function of radiation dose to obtain the cell survival curve.

RESULTS AND DISCUSSION

Pertinent toxicity data for the drugs tested are presented in Table 1. This includes the LD-50 for i.p. and p.o. administration as mg of drug per kg of animal weight (mg/kg). The dosage of drug used in radiation experiments was generally two-thirds of the toxic LD-50 value unless precluded by increased toxicity during the irradiation procedure. If this occured, subsequent use of the drug was at one-half of the previously determined toxic LD-50. Use of the nylon tubes (described above) in neutron irradiation procedures sometimes resulted in animal lethalities which were unexpected as related to the earlier drug toxicity data. These lethalities (which were especially prevalent during the summer months) were probably due to the physical and physiological constraints inherent in the use of these tubes. Following the switch to the better-designed butyrate tubes far fewer "tube" lethalities were encountered.

1. Radiation Lethality Experiments

A. Control (Untreated)

The LD-50(6) in the control animals for fission neutron irradiation was determined to be 252 rads. The 95% confidence limits were 239 to 266 rads. This is identical to the value previously reported in a similar study (24). The LD-50(30) for fission neutrons was 240 rads with 95% confidence limits of 217 rads to 264 rads as determined by probit analysis. The proximity in values for the LD-50(6) and LD-50(30) was not unlike the results of earlier studies (24), the ratio of LD-50(6) to LD-50(30) being 1.05. This demonstrates that with fission neutrons the dose needed to kill mice by the bone marrow syndrome and that necessary to kill by the gastrointestinal syndrome are not greatly different, perhaps, in this case by a factor which is nearly unity. This suggests that a relatively high RBE for gastrointestinal damage, but not for hematopoietic damage, would introduce gastrointestinal deaths in the thirty day lethal dose range used to define hematopoietic death following whole-body exposure. This phenomenon is illustrated in figures 2-3 where the daily mortality is shown as a function of time following irradiation. With the low LET cobalt-60 irradiation it is clearly seen that there is a peak of lethality prior to the six-day cut-off point used as the demarcation between gastrointestinal and hematopoietic lethality. From six to thirty days after irradiation there is a distribution of mortality which is somewhat skewed to the left (skewness = 1.5963, kurtosis = 1.7308). Following fission neutron irradiation, however, the daily mortality distribution is somewhat different. As can be seen in figure 3, there is a broad peak of lethality around the six day demarcation, occurring out to nine days following exposure to radiation. After nine days the distribution is somewhat different than that seen with gamma irradiation (skewness = 0.6387, kurtosis = -1.3388), indicating that deaths due to gastrointestinal

Fig. 2 - Daily mortality (normalized to 100)
following Co-60 irradiation

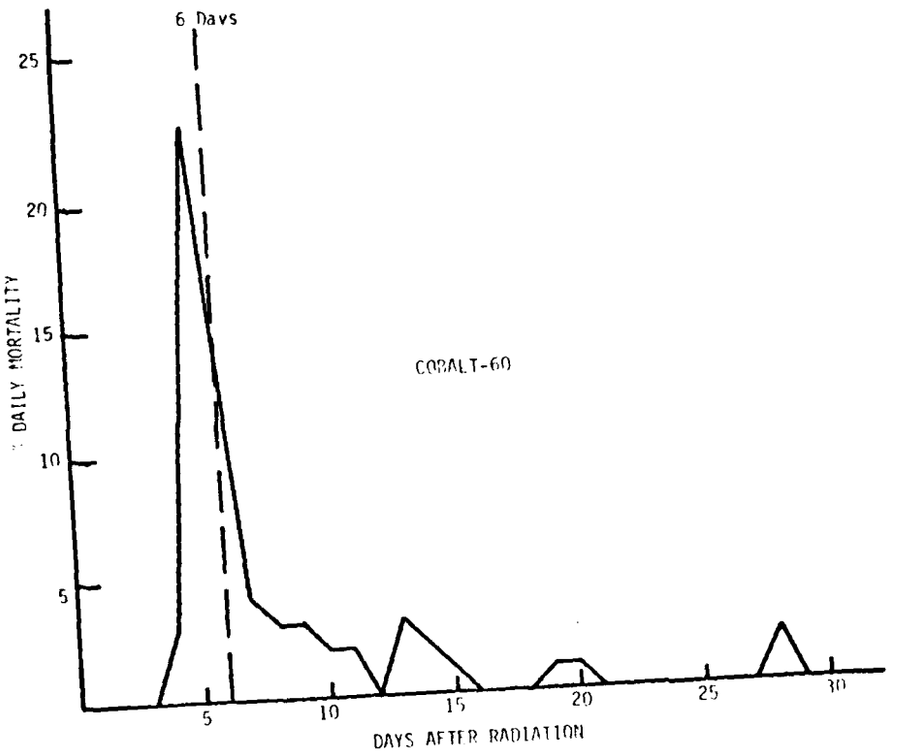
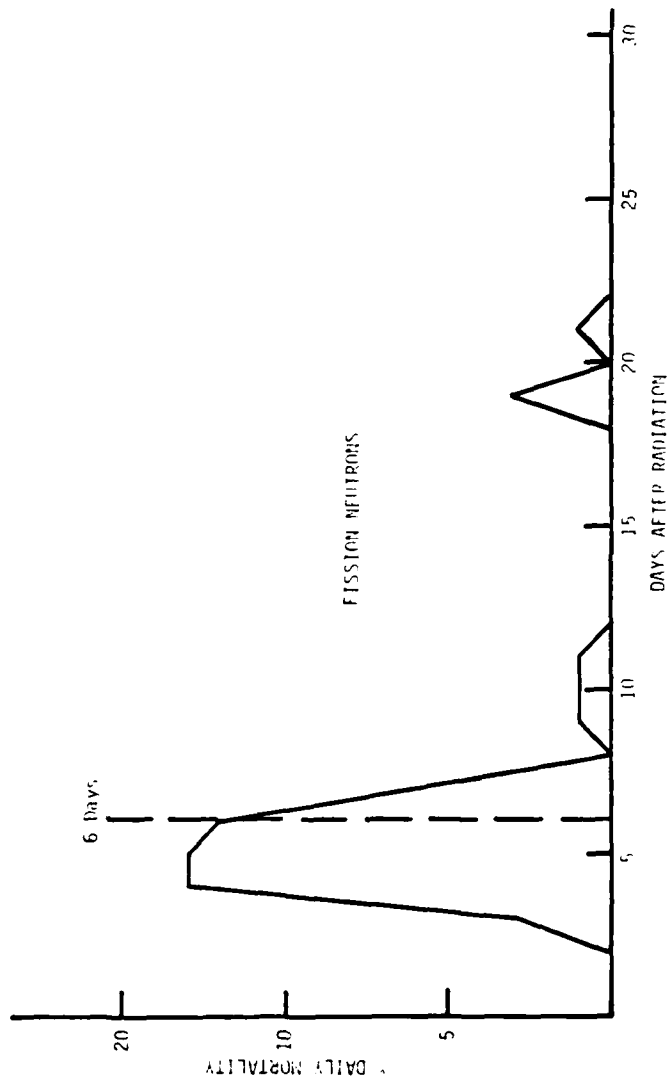

6 Days
25
20
15
10
5
DAILY MORTALITY
COBALT-60
5
10
15
20
25
30
DAYS AFTER RADIATION

Fig. 3 - Daily mortality (*normalized to 100%*)
following fission neutron radiation


FISSION NEUTRONS
DAYS AFTER RADIATION
DAILY MORTALITY
6 Days
5
10
15
20
25
30
5
10
20

causes may be occurring later than six days following fission neutron irradiation.

The LD-50(6) for Co-60 radiation was 1065 rads. This is somewhat lower than the previous study, which used 4 MeV X-rays (22), but can perhaps by accounted for by the fact that female C57B1/6 mice were used in these experiments instead of the male C57B1/6 mice used before, there being a significant difference in the radioresponse of mice of the same strain to low LET radiation. There may also be a differential response due to the different energies of the radiations employed. The LD-50(30) for Co-60 irradiation was 738 rads and the ratio of LD-50(6):LD-50(30) was 1.47.

The RBE (relative biological effectivenss) for the LD-50(6) was calculated as the ratio of the LD-50(6) for X-rays to the LD-60(6) for neutrons. This resulted in an RBE of 4.23 for death in the gastrointestinal lethality dose range. A similar calculation for LD-50(30) resulted in an RBE of 3.08, which represented death in the hematopoietic syndrome dose range. The phenomenon of a higher RBE for gut death as compared to marrow death, coupled with the low LD-50(6):LD-50(30) ratio for neutrons suggests a greater sensitivity of the intestine to neutrons than to gamma radiation, a fact which has been alluded to, but not explained, in earlier studies (23,24).

In addition to the lethality studies performed with fission neutrons and Co-60 gamma rays other information was gleaned from the hematopoietic and gastrointestinal lethality experiments. When the mean survival time is plotted as a function of radiation dose, curves such as those seen in figure 4 are obtained. Delineated in this figure are the respective survival time versus radiation dose curves for both fission neutrons and gamma rays. The portions of the curves used to obtain LD-50's for gastrointestinal and hematopoietic lethalities are shown to have a considerable overlap, especially with fission neutrons. This phenomenon and the proximity of the LD-50's for gastrointestinal and hematopoietic death again illustrate the points discussed above with regard to differences in lethality analysis found in comparisons of the effects of high LET and low LET radiation. This examination of survival time was extended with two experiments performed in the reactor pulse mode. These experiments were intended to elucidate mechanisms of lethality at higher radiation doses in order to determine if radioprotective drugs could be useful over a wide range of radiation doses and against different radiation lethality syndromes. Figure 4 shows the survival time curve when fission neutron doses of up to 8700 rads were added to those already seen in figure 3. The striking thing about these results is that even at the highest doses there is no indication of entry into the central nervous system (CNS) syndrome. Fission neutron radiation showed a RBE on the order of three to four for hematopoietic and gastrointestinal death. Previous studies indicate that the LD-50 for CNS death in the mouse is approximately 25,000 rads. Applying and RBE of three to four suggests that the LD-50 for CNS lethality with fision neutrons should be in the range of 4000 to 6000 rads. The data shown in figure 4 clearly indicates that this is not the case and

Fig. 4 - Survival time in hours as a function of radiation dose

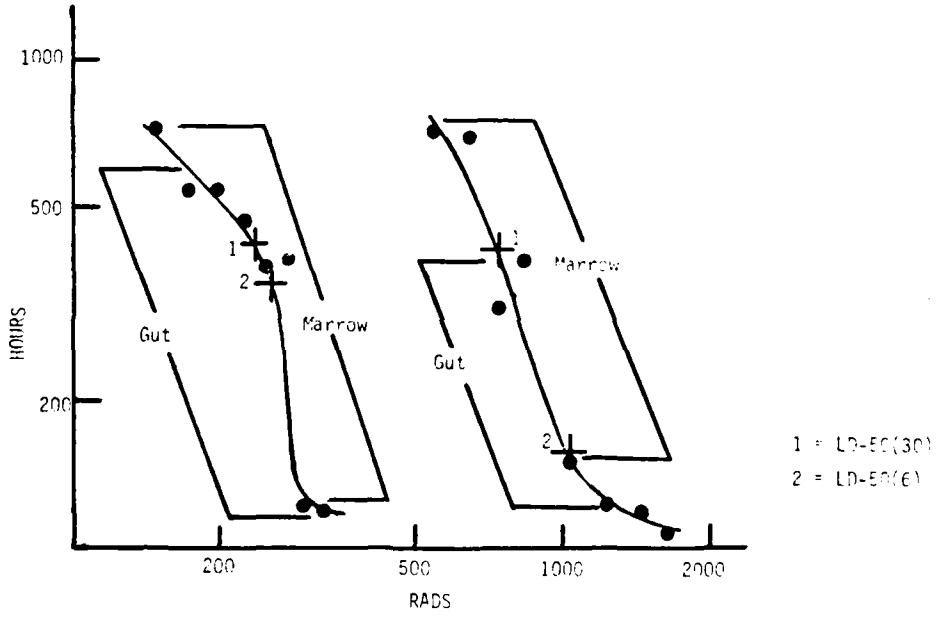

1000
500
200
HOURS
Gut
Marrow
1
2
Gut
Marrow
1
2
200
500
1000
2000
RADS
1 = LD-50(30)
2 = LD-50(6)

Fig. 5 - Survival time in hours as a function of neutron dose. Linear portions of the curve were fitted by least squares regression analysis.

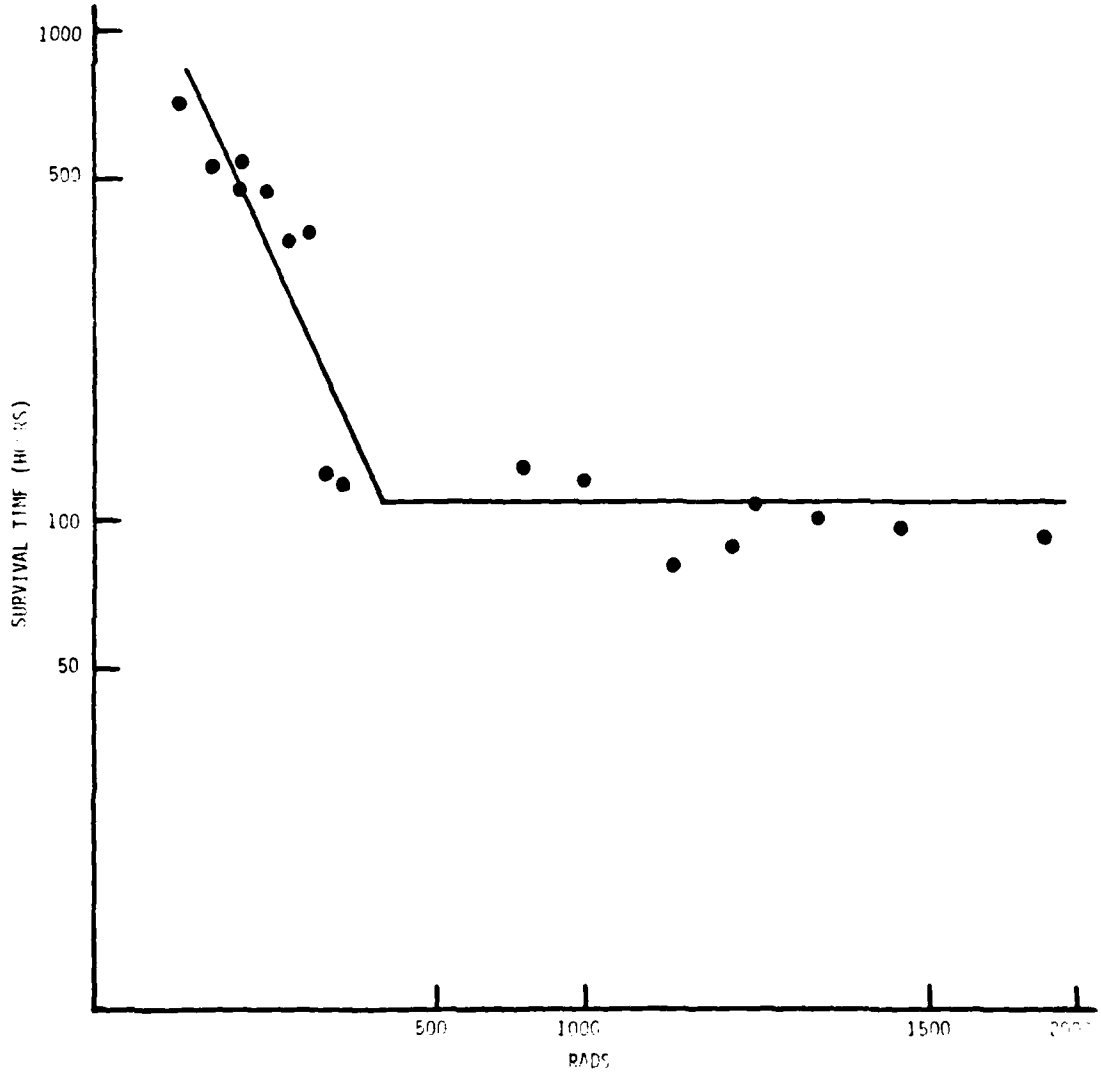

1000
500
100
50
SURVIVAL TIME (HOURS)
500
1000
1500
RADS

Table 1 Drug Information

Drug	Structure	Vehicle	LD50 (mg/kg)* i.p.	p.o.
WR 347	$NH_2Ch_2-CH_2-SH$	H_2O	498 (457-542)	
WR 2529	$NH_2CO(CH_2)_2NH(CH_2)_2SH$	H_2O	2901	
WR 2721	$NH_2(CH_2)NH(CH_2)\ SPO_3H_2$	H_2O	1108 (1064-1154)	1301 (1135-1491)
WR 3689	$CH_3NH(CH_2)\ NH(CH_2)_2SPO_3H_2$	H_2O	1449 (1378-1525)	1816 (1520-2110)
WR 44923	$NH_2(CH_2)_3NH(CH_2)_3SPO_3H_2$	H_2O	773 (583-1024)	
WR 109342	 $-CH_2NHCCH_2SH$ (NH)	H_2O	371 (35.2.39.0)	58.0 (48.6-70.8)
WR 151327	$CH_3NH(CH_2)_3NH(CH_2)_3SPO_3H_2$	H_2O	1011 (983-1039)	
WR 168643	$NaOS(CH_2)_4SSS(CH_2)_4SONa$	H_2O	1272 (1255-1290)	1142 (999-1278)
WR 176542	$NH_2(CH_2)_4CHNH_2CH_2SPO_3H_2$	H_2O	649 (629-671)	

*Values in parentheses are 95% confidence limits

Table 2 Lethality - Neutrons

DRUG	Route	Dose (mg/kg)	LD50(6)* (RADS)	LD50(6) DMF	LD50(30)* (RADS)	LD50(30) DMF
None		--	252(239-266)	--	240(226-252)	--
WR 1065	i.p.	251	358(102-601)	1.42	250(98-395)	1.04
WR 2529	i.p.	1302	370(336-403)	1.47	327(336-408)	1.40
WR 2721	i.p.	741	351(343-358)	1.39	287(280-293)	1.20
WR 3689	p.o.	1216	343(246-439)	1.36	250(-531-1031)	1.04
WR 44923	i.p.	517	447(422-472)	1.77	293(247-339)	1.22
WR 109342	p.o.	38	371(323-420)	1.47	290(264-315)	1.21
WR 151327	i.p.	677			321(300-343)	1.34
WR 168643	i.p.	852	310(274-346)	1.23	300(198-401)	1.25
	p.o.	765	330(303-374)	1.31	332(-101-708)	1.38

* Values in parentheses are 95% confidence limits; where no confidence limits appear the LD50 was estimated by linear regression.

that the RBE for CNS death following fission neutron irradiation is considerably lower than that observed for other radiation syndromes.

Radiation lethality experiments were performed with the following drugs in order to determine the protective effects of the compounds against the lethal response of either the gastrointestinal (six day) or hematopoietic (thirty day) radiation syndrome: WR 1065, WR 2529, WR 2721, WR 3689, WR 44923, WR 109342, WR 151327, and WR 168643. All pertinent results of the radiation lethality experiments may be found in table 3.

B. WR 1065

WR 1065 is the dephosphorylated metabolite of WR 2721. AS such, the deprivation of the phosphate group renders this compound much more toxic than WR 2721. The toxic LD50 for WR 1065 following i.p. injection was determined to be 375 mg/kg in our studies, versus 1108 mg/kg for WR 2721. This small structural change apparently has no implications which affect the radioprotective properties of the drug as related to gastrointestinal radioprotection. The LD-50(6) for mice injected with WR 1065 prior to whole-body fission neutron irradiation was 358 rads. Compared to the control value of 252 rads this gives a dose modification factor (DMF) of 1.42 as contrasted to the DMF of 1.39 for WR 2721 (vide *infra*) found for protection against gastrointestinal lethality. However, the LD-50(6) value found for WR 1065 is subject ot some question due to the fact that the experiment designed to determine the LD-50(6) and LD-50(30) for this drug was flawed due to premature reactor shutdown ("scram") for several of the radiation doses designed to be tested. Therefore, the probit analysis performed on the lethality results of WR 1065 contained only the four highest doses (LD50-6) and the three lowest doses (LD50-30). Because of the lack of weighing of very low response data or very high response data in the probit method of lethality analysis, the inherent error in the LD-50 determination is very large in this particular case.

WR 1065 did not protect very well against lethality due to the hematopoietic syndrome, the LD-50(30) being 250 rads as compared to the previously determined control value of 240 rads, giving a DMF of 1.04. However, the same caveat must apply to the LD-50(30) results as applies to the LD-50(6) results due to the unexpected reactor "scram."

C. WR 2529

The neutron LD-50(6) for WR 2529 following an i.p. injection of 1944 mg/kg (two-thirds of the toxic LD-50) was determined to be 370 rads. This value was calculated by probit analysis and the 95% confidence limits were determined to be 336 rads to 403 rads. From the studies performed in the previous year of this contract the LD-50(6) for mice receiving no radioprotective compound was shown to be 252 rads with 95% condidence limits of 239 to 266 rads. Therefore the dose modification factor (DMF) for this compound was 1.47, thus making it

one of the better drugs which we have assayed for protective efficacy against the neutron-induced gastrointestinal syndrome.

The LD-50(30) for mice injected with WR 2529 prior to whole-body fission neutron irradiation was found to be 327 rads (95% confidence limits = 336 - 403 rads). When this value is compared to the control value for hematopoietic lethality from the earlier study the DMF is calculated as 1.4, again showing that WR 2529 is one of the better compounds which we have found to protect against neutron radiation.

D. WR 2721

This drug is generally considered to be the best radioprotective compound yet discovered. Because of the relatively low protective effects found with WR 2721 in the first year of this study, the gastrointestinal and hematopoietic lethality studies were repeated with a wider range of doses. In all experiments the i.p. dose of WR 2721 injected was 741 mg/kg, two-thirds of the toxic LD-50 of 1108 mg/kg. The results of several experiments resulted in a redefining of the WR 2721 LD-50(6) as 351 rads (95% confidence limits = 343 - 358 rads), compared to the previously reported value of 318 rads. The resultant DMF was 1.39, thus substantiating WR 2721 as one of the better radioprotectors. Similar reassessment of results occurred when the LD-50(30) for WR 2721 was repeated. The DMF for hematopoietic death was 1.2, as calculated from a LD-50(30) of 287 rads (95% confidence limits = 280 - 293 rads), an increase of 20 rads over the aforedetermined value of 267 rads.

E. WR 3689

The toxic LD-50 for WR 3689 following per os (p.o.) administration was found to be 1815 mg/kg. Accordingly two-thirds of this dosage, or 1216 mg/kg, was used in the radiation lethality experiments. The LD-50 for gastrointestinal death was 343 rads (95% confidence limits = 246 - 439 rads, DMF = 1.36), a value not significantly different from the LD-50(6) reported for i.p. administration of the same drug (337 rads). However, the p.o. LD-50 value displays a rather large confidence interval. This is due to the fact that there were unexpected drug toxicities encountered during the fission neutron irradiation experiment with resultant decremental effect on the numerical composition of the radiation dosage groups. The same effect obtained in the LD-50(30) results, where the LD-50 was computed to be 250 rads (DMF = 1.04) with 95% confidence limits of -531 to +1031 rads, obviously a statistically flawed result. Therefore the p.o. lethalities for WR 3689 are being repeated.

F. WR 44923

Radiation LD-50 values for WR 44923 were previously reported, but due to paucity of data these experiments were repeated in the present series. Using an i.p. dose of 517 mg/kg, an LD-50(5) of 447 rads (95% confidence limits = 422 - 472 rads) was obtained. This value gave a

DMF of 1.77, by far the best protection conferred by any of the drugs being tested in the current protocols. Reconfirmation of this result is presently being planned. However, the LD-50(30) was found to be 293 rads (95% confidence limits = 247 - 339 rads), which gave a DMF of only 1.22. This result is not unexpected however, as there does not seem to a correlation between gastrointestinal and hematopoietic protective effects conferred by these compounds following fission neutron irradiation. This may be due to the phenomenon discussed above, where the differing radiosensitivities found for the two modes of lethality are not as great for fission neutron radiation as they are for low LET (gamma) radiation, therefore resulting in either a lowered resistance to gastrointestinal death or a greater resistance to hematopoietic death.

G. WR 109342

WR 109342 is an extremely toxic drug, the i.p. LD-50 being 37.1 mg/kg and the p.o. LD-50 being 58.0 mg/kg. Attempts to assay the gastrointestinal and hematopoietic protective effects of this drug following i.p. injection were unsuccessful due to toxicity problems which may have been exacerbated by use of the formerly used nylon mouse tubes which allowed little ventilatgion and therefore build-up of physiological exhalants. The p.o. assay of WR 109342 following administration of a dose of 38 mg/kg was more successful, resulting in an LD-50(6) of 371 rads (95% confidence limits = 323 - 420 rads, DMF = 1.47) and an LD-50(30) of 290 rads (95% confidence limits = 264 -315 rads, DMF = 1.21).

H. WR 151327

Toxicity data indicated an i.p. LD-50 of 1011 mg/kg, thus making WR 151327 one of the less toxic of the compounds tested in this series. Two-thirds of the LD-50, or 677 mg/kg was the dosage used in all radiation evaluations of WR 151327. Fission neutron radiation lethality experiments were performed in order to determine the radioprotective efficacy of this drug. The results for protection against the gastrointestinal radiation syndrome were inconclusive as at dosages as high as 450 rads only 25% lethalities were recorded. Because of the lack of response a probit analysis could not be computed, but the data indicate a possible LD-50 in excess of 600 rads. Therefore this data on WR 151327 is being followed up and expanded. A LD-50(30) for WR 151327 was obtained and determined to be 321 rads (95% confidence limits = 300 - 343 rads). This provides a DMF of 1.34 for protection against hematopoietic death, thus again showing no correlation between protection against gastrointestinal death and hematopoietic death.

I. WR 168643

The toxic LD-50 for this compounds following i.p. injection was 1272 mg/kg and following p.o. administration ws 1142 mg/kg. Therefore the respective dosages used for i.p. and p.o. administration were 852 mg/kg and 765 mg/kg. The i.p. LD-50(6) for WR 168643 was 310 rads (95%

confidence limits = 274 - 346 rads, DMF = 1.23). The LD-50(30) for whole-body irradiaton following i.p. injection of the drug was 300 rads with 95% confidence limits showing no significant difference from the control LD-50(30) of 240 rads. Protection by WR 168643 against gut death following oral administration was limited, showing a LD-50(60) of 330 rads (95% confidence limits = 330 - 374 rads) and a DMF of 1.31. The p.o. LD-50(30) was 332 rads (DMF = 1.38).

2. Intestinal Microcolony Survival Experiments

The following drugs were assayed for their protective efficacy in the intestine utilizing microcolony survival: WR 347, WR 2721, WR 3689, WR 44923, and WR 51327. All of the drugs tested showed some protection as seen by this method, protection which was commensurate with that seen in the lethality assays.

A. Control

Animals were irradiated without the benefit of drug protection in separate experiments using both Co-60 gamma rays and fission neutrons. The resulting survival curves are shown in figure 6 and the parameters describing these curves may be found in table 3. The differences between the low LET gamma ray survival curve and the high LET fission neutron survival curve were as expected. When plotted on the same-axes the respective curves demonstrate the usual response of neutrons vis a vis gamma rays. Compared to the gamma ray response curve the neutron response curve is shifted to the left (Dq for neutrons = 205 rads, Dq for gamma rays = 907 rads) and displays a steeper slope (m = -0.0198 vs. m = -0.0093). The resultant Do's were for neutrons 50.5 rads and for gamma rays 108 rads. When the RBE is calculated as the ratio of Do's a value of 2.14 is recorded, which is substantially lower than the RBE for gastrointestinal death calculated as the ratio of LD-50(6)'s (RBE = 4.15). However, when the RBE is calculated as the ratio of radiation doses at a known level of survival, viz., the dose needed to reduce the number of surviving cells per circumference to 10, the RBE is 3.28, nearer the value obtained from the radiation lethality experiments. It should be noted that any discrepancy in the RBE's calculated from cell survival curves and lethality experiments likely arises from the fact that in the whole-body radiation used to induce the gastrointestinal lethality response, other factors than strictly intestinal cell death are probably components of the entire syndrome of symptoms and reactions which contribute to the ultimate gastrointestinal lethality. The measured parameter of intestinal cell survival as derived from microcolony formation is only one part of this entire complex of systemic and local physiological and histological responses.

B. WR 347

This compound showed the least radioprotection in this particular cellular assay system than any of the drugs tested in this series (figure 7). The ratio of the radiation doses needed to reduce the number of

TABLE 3

Intestinal Survival Curve Data - Neutrons

DRUG	i.p. Dose (mg/kg)	Slope	Correlation Coefficient	Do (rads)	Dq (rads)	D-10 (rads)	DMF
None	Cobalt-60	-0.0093	-0.9916	108	907	1359	
None	Neutrons	-0.0198	-0.9824	50.5	205	517	
WR 347	220	-0.0194	-0.9282	51.5	221	436	1.05
WR 2721	741	-0.0226	-0.9826	44.4	297	481	1.15
WR 3689	970	-0.0177	-0.9664	56.6	280	517	1.24
WR 44923	517	-0.0200	-0.9466	49.9	266	475	1.14

surviving cells per circumference to 10 (treated:untreated, hereafter termed the D-10) was 1.05, significantly lower than the DMF of 1.39 obtained through lethality assays, although this difference may be accounted for by the reasons stated above. The slope for the WR 347 microcolony survival curve (m = -0.0194) is not significantly different from that obtained from the untreated control animals, thereby exhibiting parallelism, indicating a single mechanism of protection. For WR 347, the correlation coefficient (r) was -0.9282, the Do was 51.5 rads, the Dq (quasi-threshold dose) was 221 rads, and the D-10 was 436 rads.

C. WR 2721

Using the rad difference at the D-10 level, the DMF for WR 2721 as determined by the microcolony assay system was 1.15, again not as great as the DMF determined by lethality (DMF = 1.39), but within the bounds of error as imposed by the respective assay systems and discussed above. The slope (m = -0.0226) was not significantly different from the control slope (m = -0.0198) and demonstrated significant linearity (r = -0.9826). The Do was 44.4 rads, the Dq was 297 rads, and the D-10 was 481 rads (figure 8).

D. WR 3689

This compound exhibited moderate protection against gastrointestinal radiation damage, showing a DMF of 1.24 as determined by the ratio of the D-10's. The slope was -).0177 (non-significantly different from controls), the Do was 56.6 rads, and the quasi-threshold dose (Dq) was 280 rads. The correlation coefficient (r) was -0.9664, indicating a significant degree of correlation between surviving cells per circumference and neutron dose. The D-10, used to calculate the dose modification factor, was 517 rads (figure 9).

E. WR 44923

WR 44923 demonstrated a degree of protection in this assay system which was unremarkable in its difference from the other compounds tested in the current series. The DMF was 1.14 with a D-10 of 475 rads. The slope was -0.0200, no different from controls, the Do was 49.9 rads, the correlation coefficient was -0.9466, and the Dq was 266 rads (figure 10).

F. WR 151327

The intestinal microcolony assay for this drug is currently in progress.

3. Endogenous Spleen Colony Assays

Experiments to determine to degree of neutron radiation damge to the blood-forming organs were performed utilizing the endogenous spleen colony assay. These studies were done on untreated (control)

Fig. 6 - Intestinal Cell Survival - Control

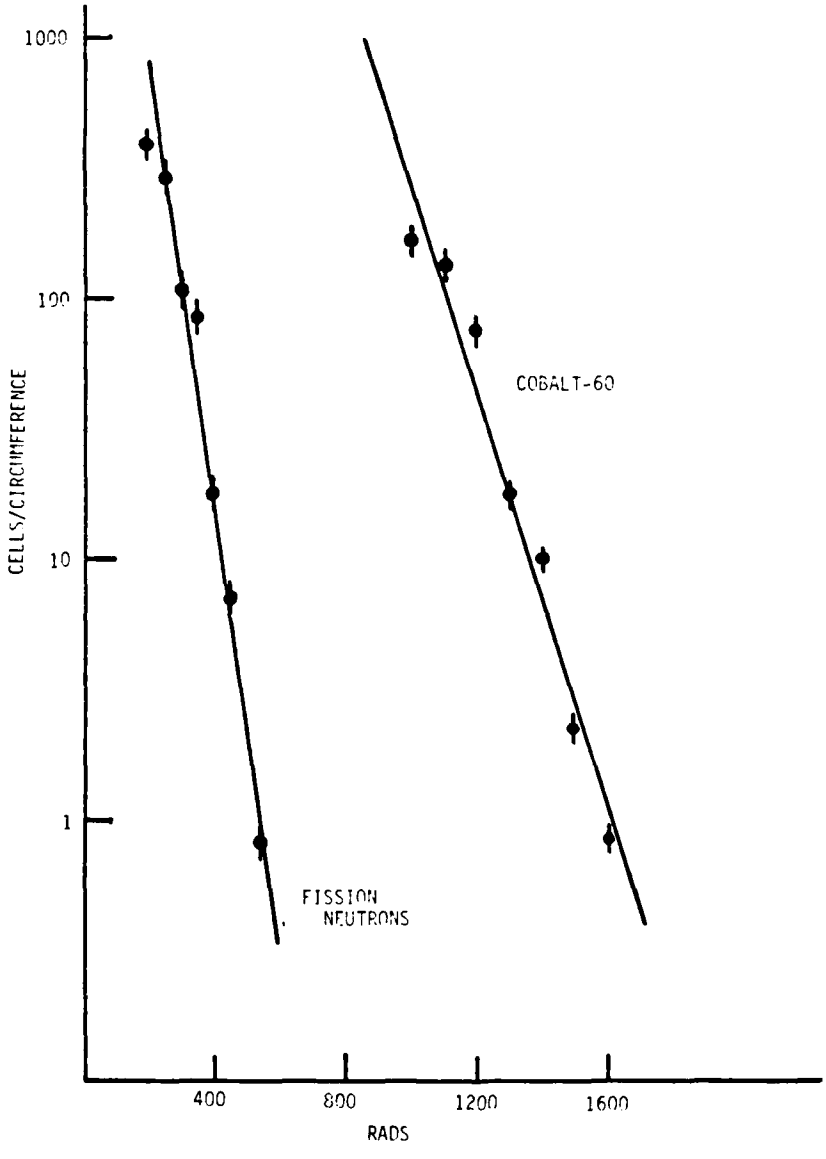
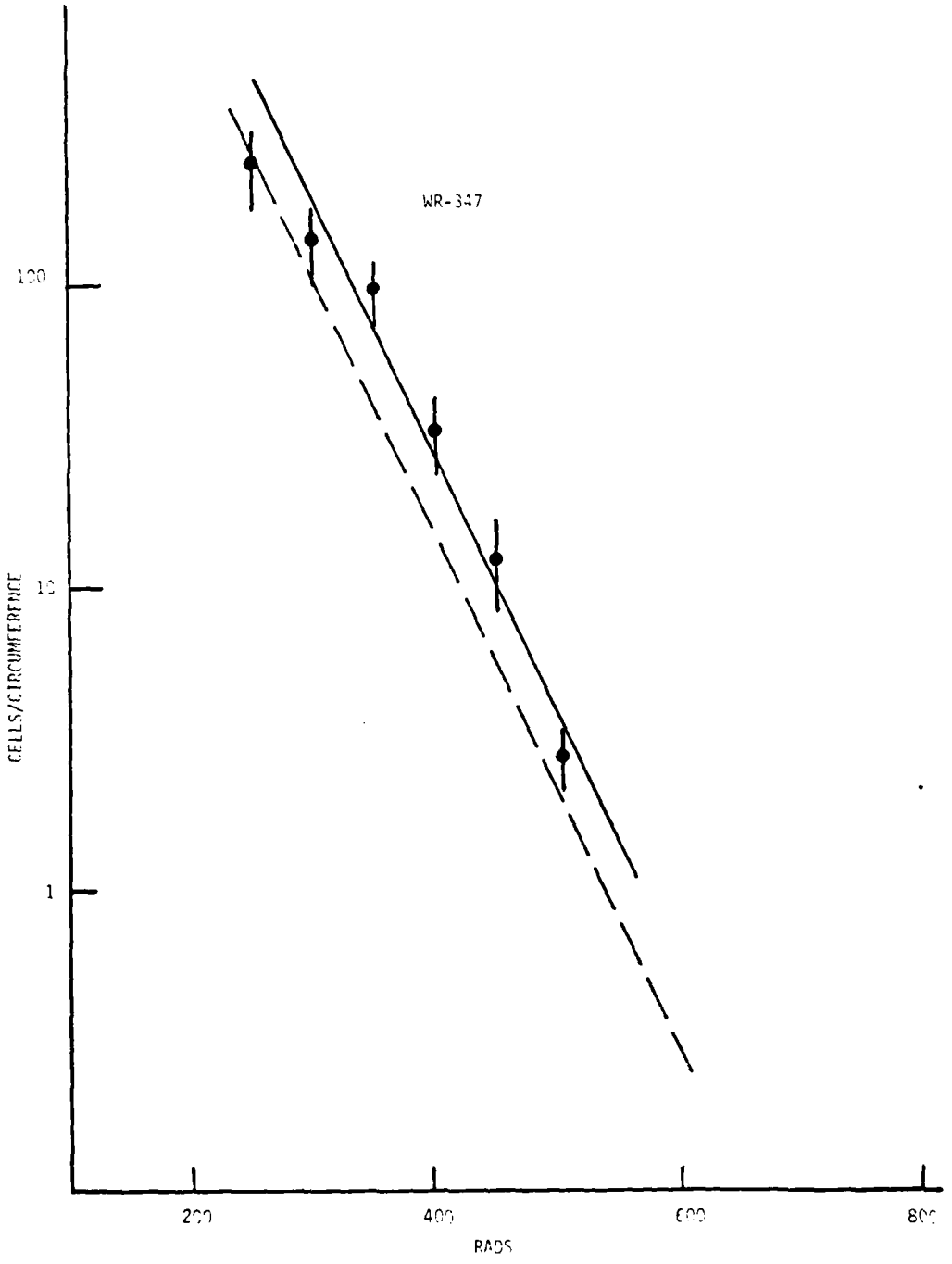

1000
100
10
1
CELLS/CIRCUMFERENCE
COBALT-60
FISSION
NEUTRONS
400
800
1200
1600
RADS

Fig. 7 - Intestinal Cell Survival - WR 347


WR-347
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CELLS/CIRCUMFERENCE
200
400
600
800
RADS

Fig. 8 - Intestinal Cell Survival - WR 2721

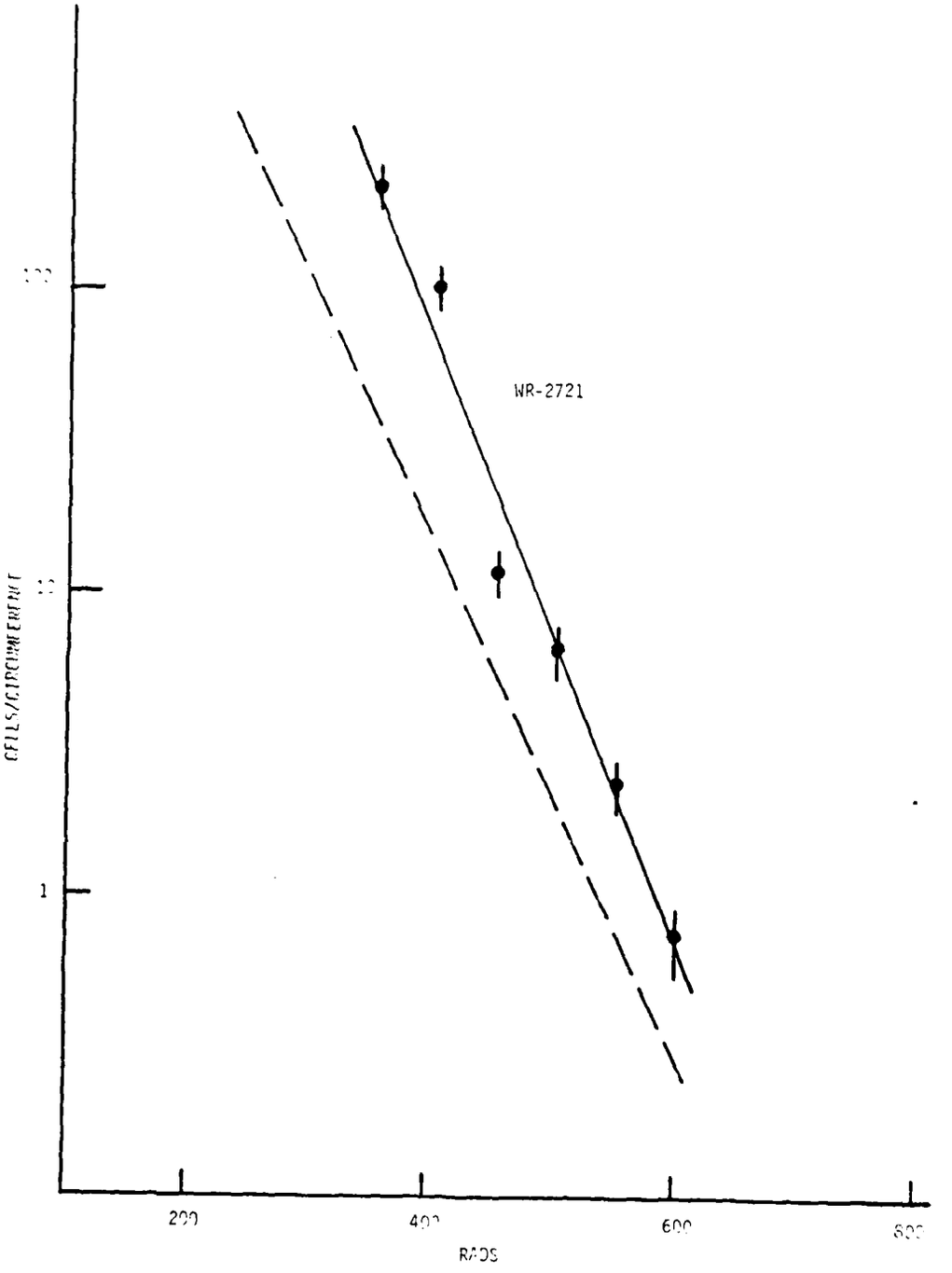

WR-2721
1
200
400
600
RADS

Fig. 9 - Intestinal Cell Survival - WR 3689

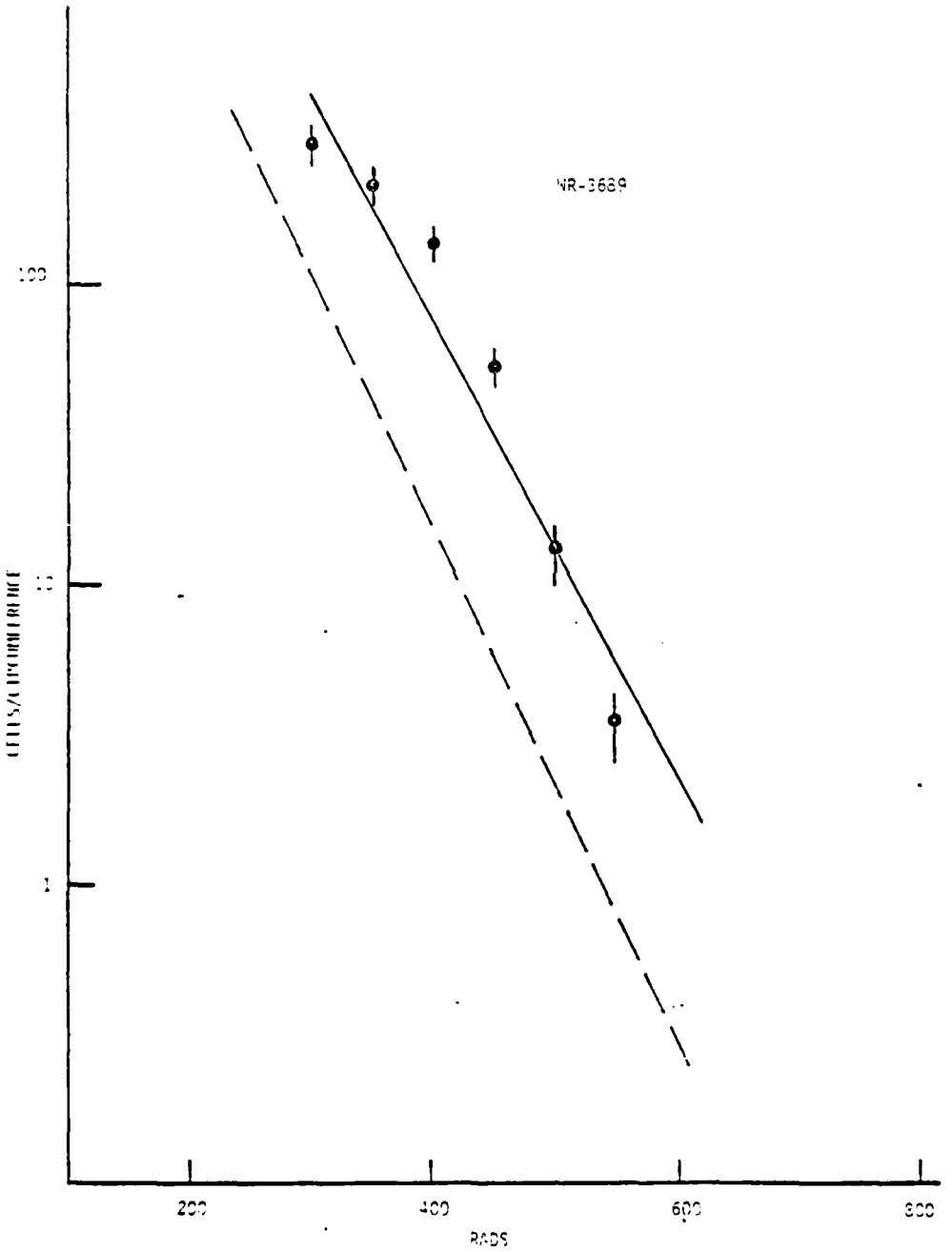
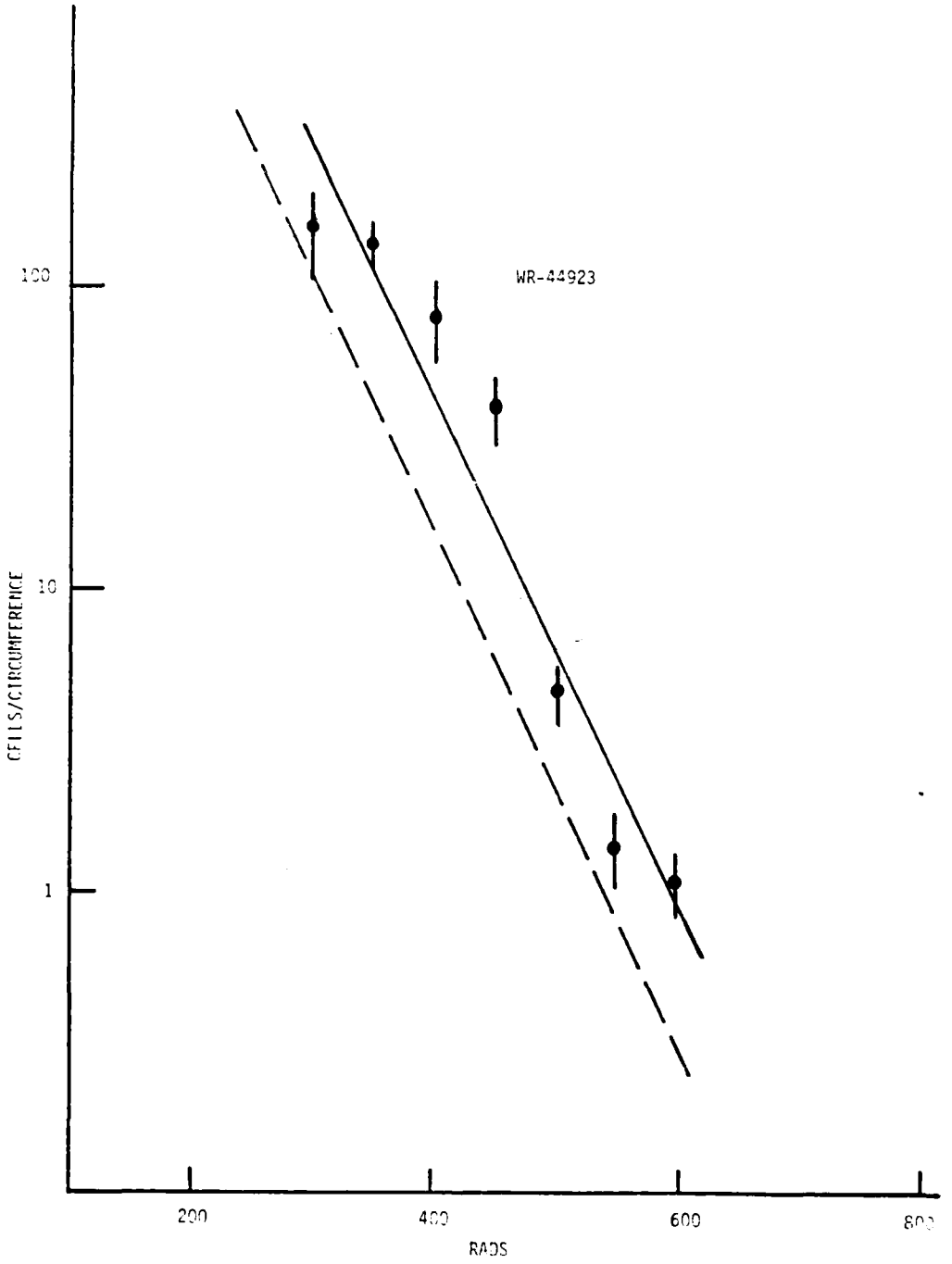

WR-3689
100
10
1
200
400
600
800
RADS

Fig. 10 - Intestinal Cell Survival - WR 44923


100
10
1
CELLS/CIRCUMFERENCE
WR-44923
200
400
600
800
RADS

animals following either fission neutron or Co-60 gamma radiation and with the following drugs following fission neutron radiation: WR 347, WR 2529, WR 2721, WR 3689, and WR 44923. Table 5 lists the parameters of the dndogenous spleen colony survival curves.

A. Control

Untreated mice weere irradiated with doses of Co-60 gamma radiation ranging from 450 to 1000 rads. This resulted in a decremental response over three decades of transformed spleen colony counts, from approximately 20 to 0.2 colonies per spleen. There was a high degree of correlation between colonies/spleen and radiation dose, with the correlation coefficient equivalent to -0.9874. The slope of the survival curve was -0.008, and the Do was 130 rads. The D-1.0 (the radiation dose needed to reduce the colony survival to an average of one per spleen) was 825 rads (figure 10).

Following fission neutron irradiation the endogenous spleen colony assay as used on untreated mice produced a survival curve with the following characterristics: correlation coefficient = -0.9974, slope = -0.029, Do = 34.83, D-1.0 = 262 rads. As expected, when plotted on the same axes as the low LET survival curve (figure 10), the neutron curve is found shifted to the left and with a significantly steeper slope. The RBE for fission neutrons, calculated as the ratio of D-1.0(gamma rays):D-1.0(neutrons) was 3.15, nearly the same as the RBE calculated for lethality due to bone marrow death. This similarity in RBE's for the two modes of response (marrow death versus spleen colony survival) indicates that the endogenous spleen colony assay is a good predictor of whole-body radiation lethality in the hematopoietic radiation dose range at the D-1.0 level.

B. WR 347

WR 347, a sulphydryl compound without a covering phosphate, provided essentially no protection using the endogenous spleen colony assay (figure 12). The dose modification factor (DMF), calculated from the ratio of the D-1.0(treated):D-1.0(untreated) was 0.939. The slope was -0.025, the Do was 40.1, and the correlation coefficient was -0.869.

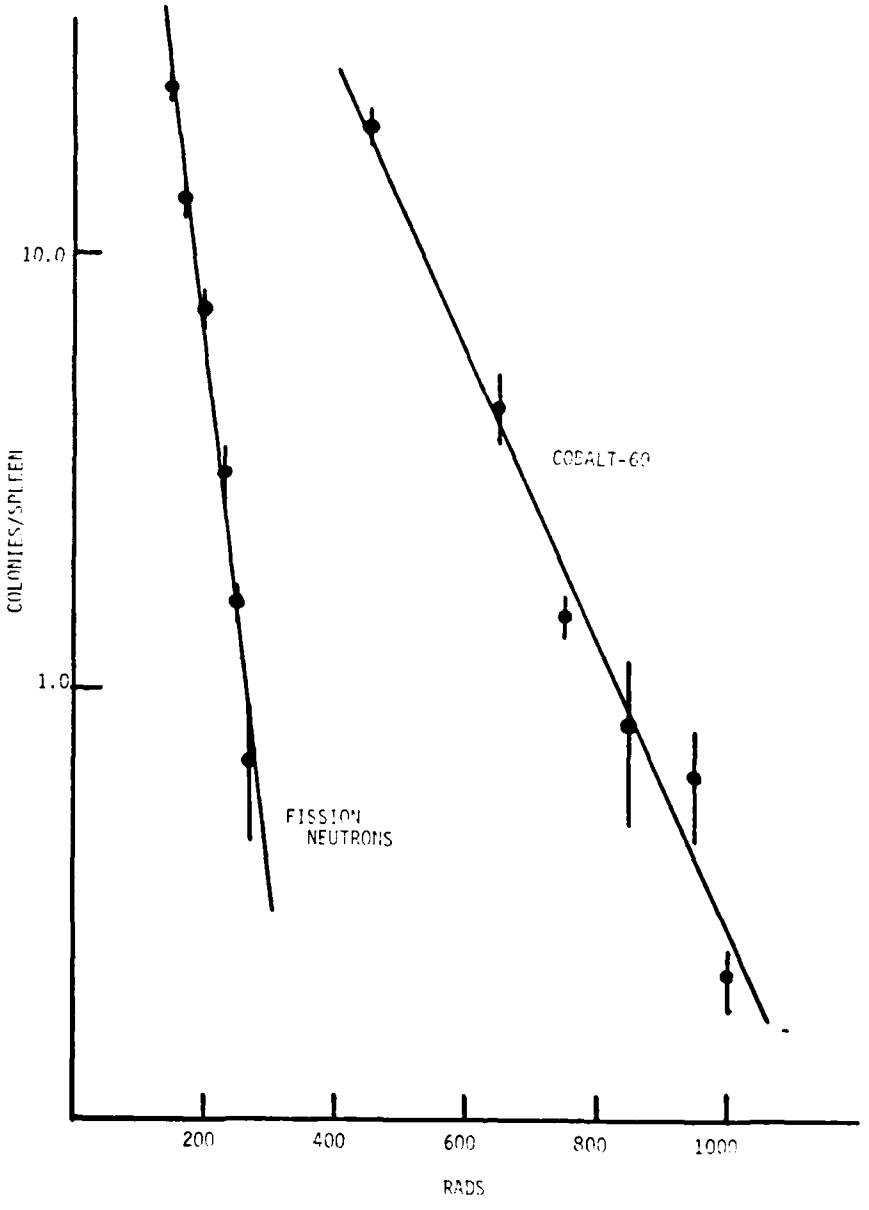
C. WR 2529

This drug exhibited a DMF of 1.15. The correlation between decremental survival and radiation dose was -0.986, the slope of the survival curve was -0.025, the Do was 40.7 rads and the D-1.0 was 302 rads (figure 13).

D. WR 2721

WR 2721 was found to have a DMF of 1.10 using this assay. Apparently for spleen colony survival following irradiation there is little correlation between the protective effect against lethality and the protective effect against blood-forming structure damage in the

Fig. 11 - Spleen Cell Survival - Controls


10.0
1.0
COLONIES/SPLEEN
COBALT-60
FISSION
NEUTRONS
200
400
600
800
1000
RADS

spleen. The correlation coefficient for the survival curve was -0.909, the slope was -0.024, the Do was 41.6 rads, and the D-1.0 was 287 rads (figure 14).

E. WR 3689

The DMF for WR 3689 was 1.18. The parameters for the survival curve were as follows: correlation coefficient = -0.986, slope = -0.027, Do = 37.3 rads, and D-1.0 = 308 rads. This drug showed the best protection of those tested with the endogenous spleen colony assay (figure 15).

F. WR 44923

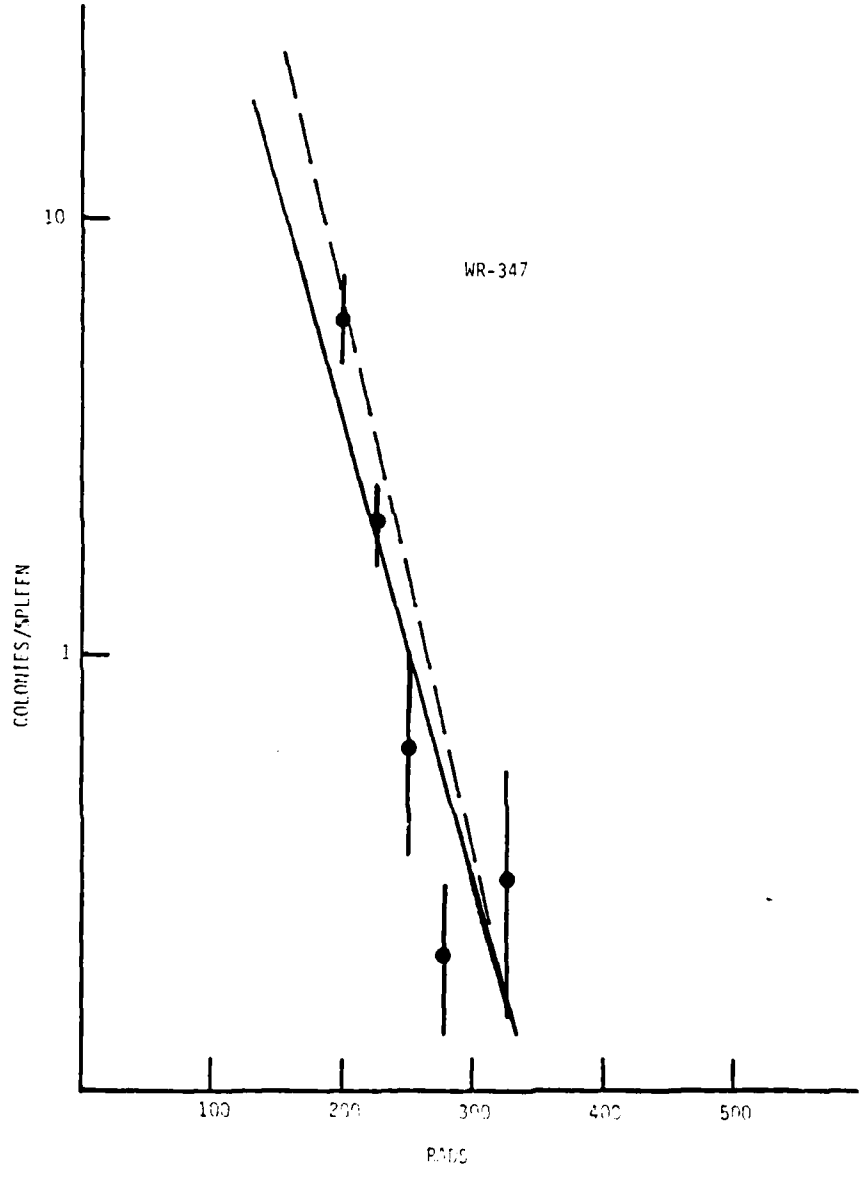
Using the D-1.0 ratio to calculate the DMF resulted in a value of 1.02 for WR 44923. The slope of the transformed spleen colony survival curve was -0.0176, the Do was56.8 rads, the correlation coefficient was -0.9337, and the D-1.0 was 267 rads (figure 16).

TABLE 4

Spleen Cell Survival Data - Neutrons

DRUG	i.p. Dose (mg/kg)	Slope	Correlation Coefficient	Do (rads)	D-1.0 (rads)	DMF
None	Cobalt-60	-0.008	-0.9874	130	825	
None	Neutrons	-0.029	-0.9974	34.8	262	
WR 347	220	-0.025	-0.8691	40.1	246	0.94
WR 2529	1302	-0.025	-0.9864	40.7	302	1.15
WR 2721	741	-0.024	-0.9092	41.6	287	1.10
WR 3689	970	-0.027	-0.9865	37.3	308	1.18
WR 44923	517	-0.018	-0.9337	56.8	267	1.02

Fig. 12 - Spleen Cell Survival - WR 347


10
1
WR-347
COLONIES/SPLEEN
100
200
300
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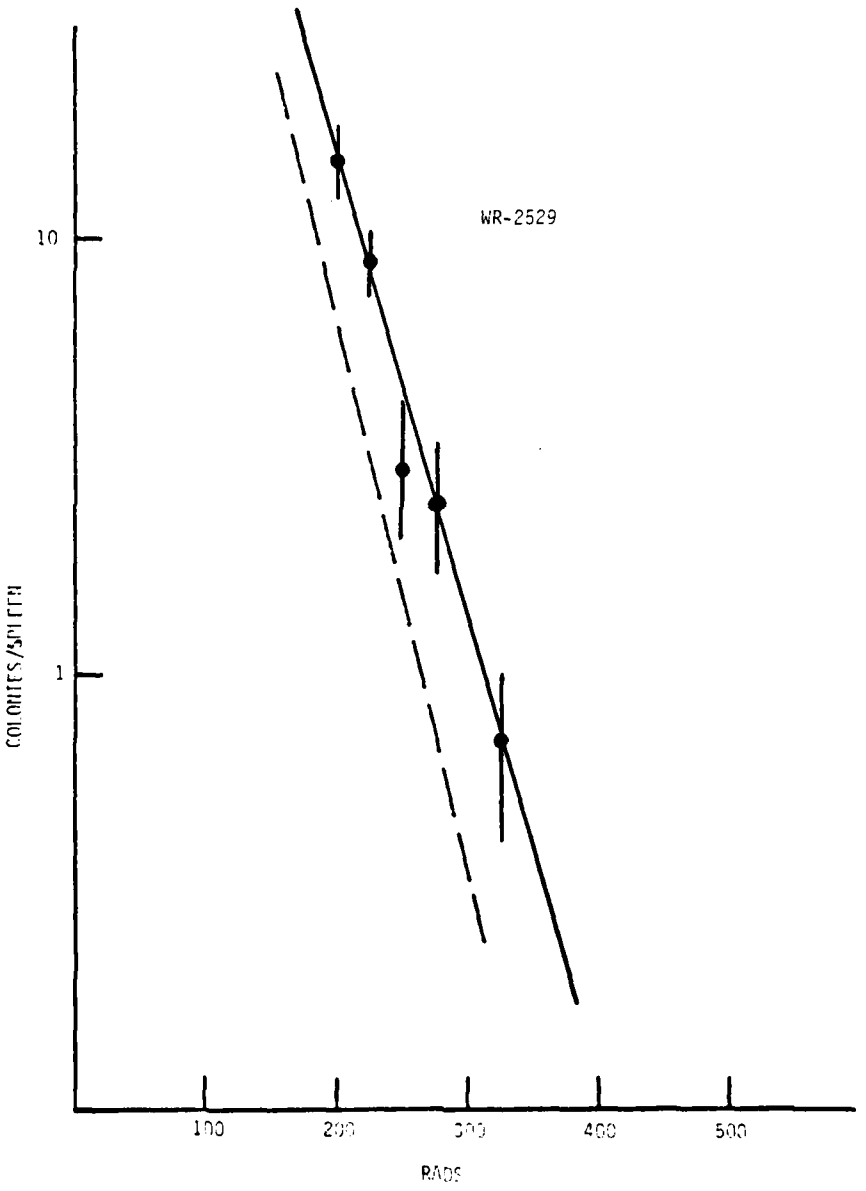
RADS

Fig. 13 - Spleen Cell Survival - WR 2529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200
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400
500
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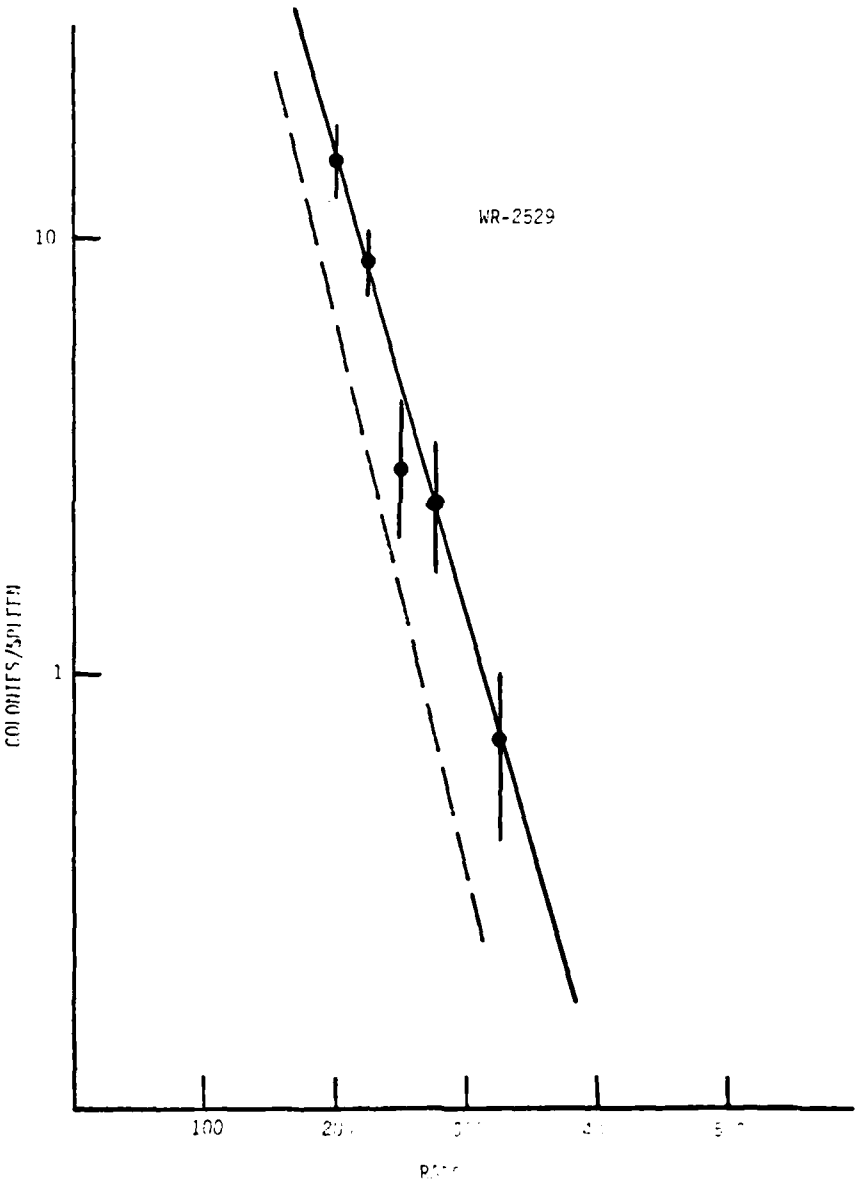
WR-2529
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1
100

Fig. 14 - Spleen Cell Survival - WR 2721

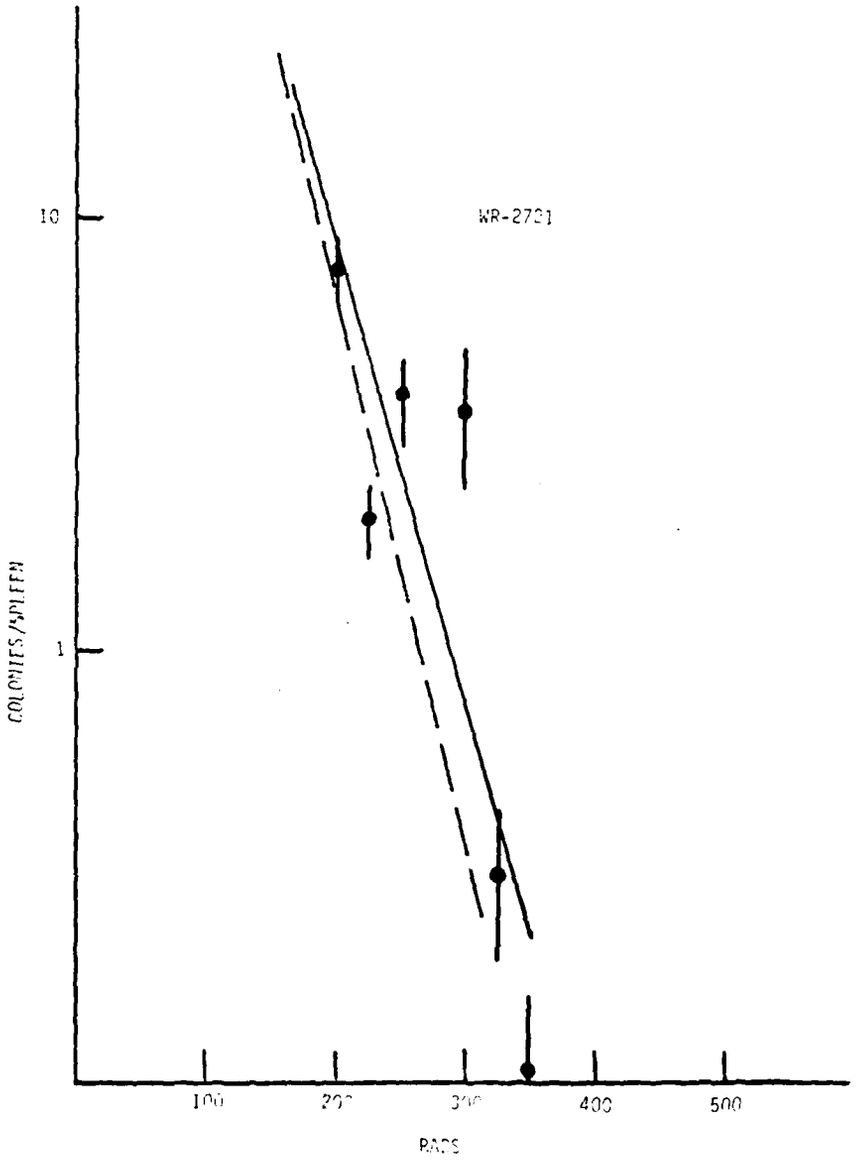
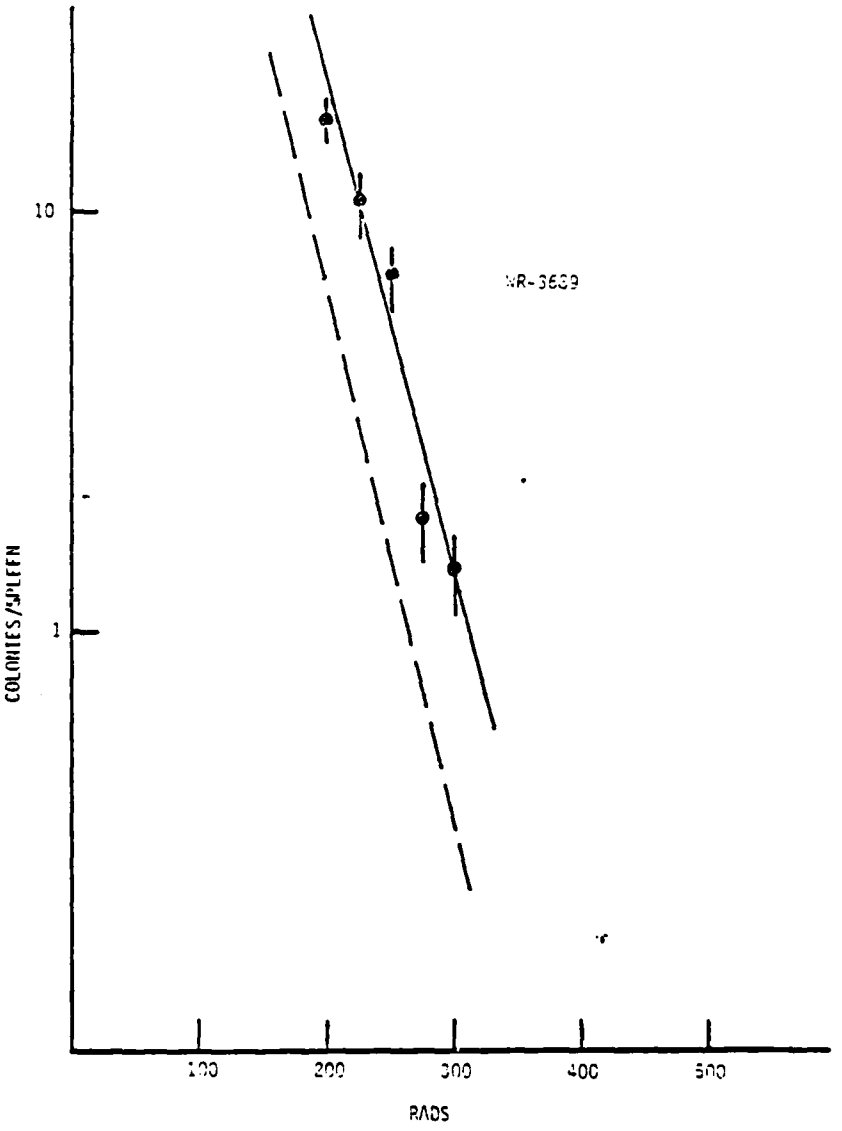

10
1
WR-2721
COLONIES/SPLEEN
100
200
300
400
500
RADS

Fig. 15 - Spleen Cell Survival - WR 3689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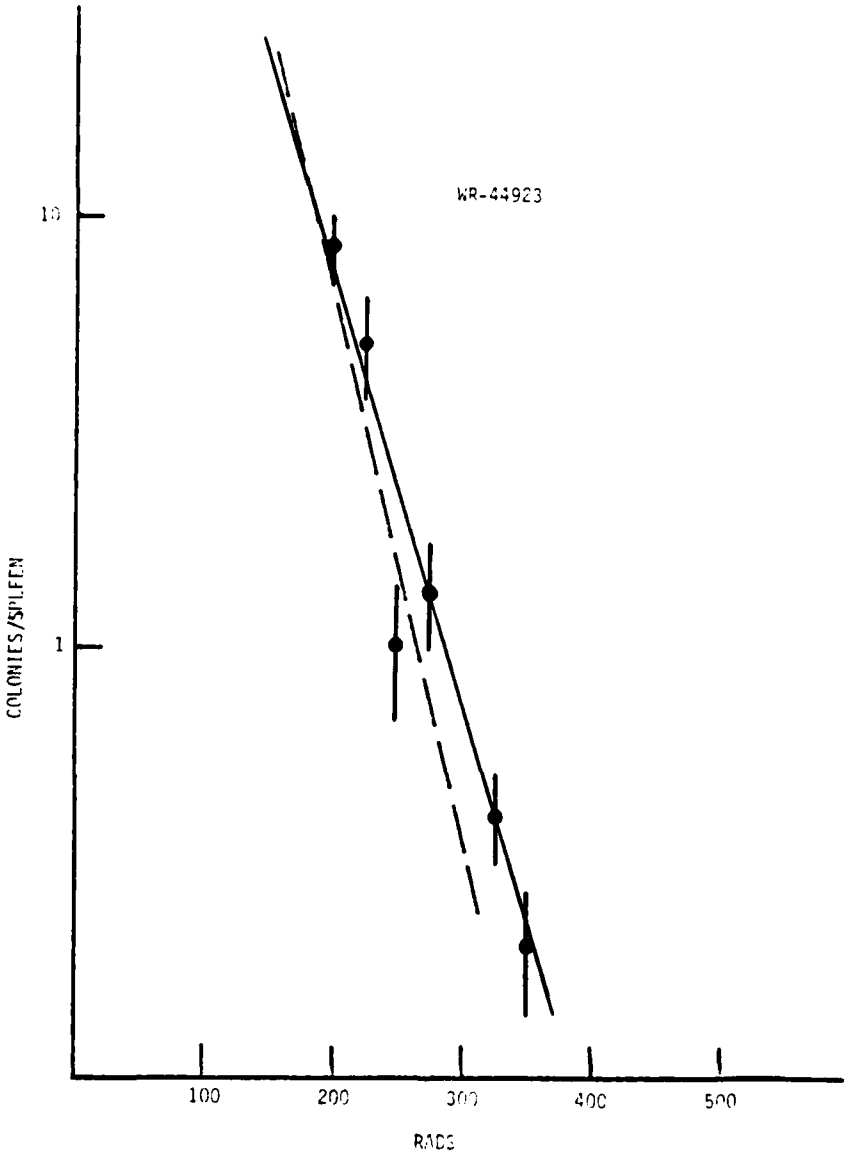
COLONIES/SPLEEN
WR-3689
100
200
300
400
500
RADS

Fig. 16 - Spleen Cell Survival - WR 44923


WR-44923
10
1
COLONIES/SPLEEN
100
200
300
400
500
RADS

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Fission, neutrons, Co-60, gamma radiation, mouse, lethality, radiation effects, whole-body radiation, radiation protection, intestine, bone marrow.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report describes the assays of various compounds for their toxicity and anti-radiation efficacy following exposure to either Co-60 or fission neutron irradiation. The chemical covered in this report are: WR-347 WR-1065, WR-2529, WR-2721, Wr-3689, WR-109342, WR-151327 and WR-168643.
The drugs and their respective dose modification factors (DMF) for

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fission neutron gastrointestinal lethality (LD50-6) following intraperitoneal administration are, in decreasing order of effectiveness: WR 44923 (1.77), WR 2529 (1.47), WR 1065 (1.42), WR 2721 (1.39), WR 16843 (1.23). Following per os (P. O.) administration of the drug, the DMF's for the LD50-6 are: WR 109342 (1.47), WR 3689 (1.36), and WR 168643 (1.31).

For hematopoietic neutron radiation lethality (LD50-30) the DMF's are: following i.p. administration, WR 2529 (1.40), WR 151327 (1.34), WR 168643 (1.25), WR 44923 (1.22), WR 2721 (1.20), WR 1065 (1.04); following P. O. administration, WR 168643 (1.38), WR 109342 (1.21), WR 3689 (1.04).

Using an intestinal microcolony assay system the following drugs provided the listed DMF;s against neutron radiation after i.p. injection: WR 3689 (1.24), WR 2721 (1.15), WR 44923 (1.14), and WR 347 (1.05).

The protective effects against neutron radiation using an endogenous spleen colony assay and i.p. administration were: WR 3689 (1.18), WR 2529 (1.15), WR 2721 (1.10), WR 44923 (1.02) and WR 347 (0.94).